APPENDIX 4
ABSTRACT OF GENETIC MARKING PROTOCOL

We propose to use retroviral-mediated gene transfer of the neomycin-resistance marker gene into autologous bone marrow and peripheral blood stem cells to study the biology of hematopoietic reconstitution after transplantation and the feasibility of using this type of delivery system to transfer foreign genes to short and long-term reconstituting cells. We will apply identical retroviral transduction conditions and vectors to autologous bone marrow and peripheral blood stem cells harvested for transplantation as part of three clinical protocols. These three protocols enroll patients with multiple myeloma, chronic myelogenous leukemia, or metastatic breast cancer. The protocols are being carried out in collaboration between two institutions: the National Institutes of Health (Clinical Hematology Branch, NHLBI and Medicine Branch, NCI) and the University of Virginia School of Medicine, Department of Hematology and Oncology. The decision to submit a "generic" genetic marking amendment for all three clinical protocols is based on the use of identical transduction conditions and vectors by the same group of investigators in each.

Eligible patients will have 70% of their harvested bone marrow and peripheral blood stem cells processed and cryopreserved as per the original protocols. The remaining 30% will be enriched for cells carrying the CD34 antigen, a protein found on the surface of primitive progenitor and stem cells, and transduced with Neo^R retroviral marking vector *in vitro* for a 72 hour period in the presence of hematopoietic growth factors. Myeloma and CML patients receiving both bone marrow and peripheral blood stem transplantation will have these two populations marked with two separate vectors. After conditioning chemoradiotherapy, both the transduced and the non-transduced populations will be returned to the patient. Molecular analysis for the marker gene will be carried out, and if successful short or long-term marking occurs, critical pilot information may be obtained regarding the biology of autologous reconstitution, the feasibility of retroviral gene transfer into hematopoietic cells, and the contribution of viable tumor cells within the autograft to disease relapse.